FUT1 mutations responsible for the H-deficient phenotype in the Polish population, including the first example of an abolished start codon

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Introduction

The blood group phenotype O_h (Bombay) is defined in routine blood grouping by the complete absence of A, B and H antigens on red blood cells and in body fluids, and by the presence of anti-A, anti-B and anti-H in serum[1]. This phenotype is extremely rare in Europe, where the frequency is estimated to be one case per million inhabitants[2] while the frequency of heterozygosity for a non-functional *FUT1* allele has been calculated to be 1 in 347 in the same population[3]. The phenotype arises due to inactivating alterations in two genes: *FUT1* and *FUT2*, located on chromosome 19q13.3. In the classic O_h phenotype, a missense mutation in *FUT1* (c.725T>G) is tightly linked to a deletion of *FUT2*[4,5], but many other mutations have been described[2]. The *FUT1* gene encodes an α-1,2-fucosyltransferase (α2Fuc-T1 or H transferase), which synthesises blood group H antigens on type 2 glycan chains, for instance as part of glycosphingolipids and glycoproteins on red cells. The *FUT2* (Se, secretor) gene is highly homologous to *FUT1* and its expression is necessary for the presence of A, B, and H antigens (of type 1) in plasma and other body fluids[6].

In this paper we report the serological and genetic characterisation of the O_h phenotype in three unrelated Polish blood donors.

Material and methods

Serology

Following informed consent, blood samples were drawn from three unrelated blood donors for further investigation after potent allo anti-H was identified in their plasma. Standard serological techniques were used[7]. ABO typing was performed by column agglutination (Bio-Rad Inc., Hercules, CA, USA). Determination of anti-A, anti-B and anti-H titres as well as H antigen typing with both anti-H lectin (Ulex europaeus; ImmucorGamma, Atlanta, GA, USA) and two different potent human anti-H (Donors ID: JP G156-213 and 274522, SCARF exchange) were performed by the tube test. Adsorption/elution studies using a conventional heat elution technique[7] were performed with both human polyclonal anti-H and lectin anti-H on the red blood cells from donors #1 and #2; and with human polyclonal anti-B and with H lectin only on red blood cells from donor #3[7]. Flow cytometry was performed using monoclonal anti-A (clone ES15), anti-B (clone 9621A8) and anti-H (clone BRIC231; FITC conjugated), as described previously[8,9].

Molecular analysis

Routine in-house polymerase chain reactions with allele-specific and restriction fragment length polymorphism methods were used to determine the ABO genotype[10-13]. *FUT1* and *FUT2* gene-specific primers were used to amplify genomic DNA for sequencing[14]. International Society for Blood Transfusion (ISBT) allele terminology is used to classify the *FUT* and *ABO* genotypes.

Results

Serological results in all samples were typical for the O_h phenotype: total absence of A, B and H antigens on the donors' red blood cells and the presence of strongly reactive anti-A, anti-B and anti-H in the donors' sera. All three donors' sera strongly agglutinated cells of O group cord blood (Table I). The antibody titres varied between the samples and donor #3 had a notably high anti-H titre (256). Flow cytometry confirmed the absence of A, B and H antigens in all samples. However, an eluate prepared following adsorption of donor #3 red blood cells with human polyclonal anti-B was weakly reactive with group B red blood cells (Table II).

ABO genotyping determined that donor #1 was *ABO.O*01.01/01.01*, donor #2 was *ABO.O*01.01/02.01* and donor #3 was *ABO*B.01/O.01.02* (Table III). Analysis of *FUT2* showed that all three donors were homozygous for c.428G>A, which encodes a change Trp143Ter and constitutes the most common basis for non-secretor status in Caucasians. DNA sequence analysis of the coding region of *FUT1* revealed a different and previously unpublished mutation in each of the three donors: donor #1 was homozygous for

Table I - Serological characteristics by saline-tube tests of three O_h (Bombay) individuals.

Bombay individuals	Antigens on red blood cells				Titre of antibody in sera against red blood cells			Reaction with O group cord blood
	A	B	H	Lewis phenotype	A1	B	O	
Donor #1	-	-	-	Le(a+b–)	128	32	8	3+
Donor #2	-	-	-	Le(a+b–)	32	32	8	3+
Donor #3	-	-	-	Le(a+b–)	512	64	256	4+

Table II - Results of absorption/elution studies performed for detection of B and H antigen on red blood cells from donor #3.

Adsorption/elution		Activity of eluates* with red blood cells of group:		
Red blood cells	**adsorbed with:**	**O**	**B**	**O_h**
Donor #3	anti-H	Neg	Neg	Neg
	anti-B	Neg	Pos	Neg
Group B donor (control)	anti-H	Pos	Pos	Neg
	anti-B	Neg	Pos	Neg

*Tested by saline test.

c.958G>A, which encodes a change of p.Gly320Arg; homozygosity for c.1A>C, was identified in donor #2, which encodes a change of p.Met1Leu but, more importantly, disrupts the translation-initiating start codon for the *FUT1* coding region; and lastly, donor #3 was found to homozygous for c.323G>T, a change that predicts p.Arg108Leu.

All three variants were analysed with both SIFT (http://sift.jcvi.org/) and PolyPhen (http://genetics.bwh.harvard.edu/pph2/index.shtml) software and found to be potentially damaging by at least one of the programmes (Table III).

Discussion

Over 50 different mutations in the *FUT1* gene have been identified to be responsible for weakness or absence of H antigen on red blood cells[15]. In this paper we report the identity of three previously unpublished *FUT1* alleles in individuals of Polish origin with undetectable H antigen on their red blood cells. The complete absence of H antigen from the donors' red blood cells showed that the novel *FUT1* mutations lead to the loss of H transferase activity. All three were apparently homozygous for the mutation in question; although hemizygosity together with a deleted *FUT1* gene cannot be ruled out, the consequence remains the same.

As yet, there is no three-dimensional crystal structure of the α2Fuc-T1 transferase and thus it is difficult to predict the location and effect of different mutations in the enzyme. However, analysis of the sequences of all three variants by SIFT and PolyPhen software confirmed that the observed mutations potentially damage enzymatic activity (Table III). Parallels with other glycosyltransferases would suggest that the change of p.Arg108Leu (observed in donor #3) occurs in the stem region of this type 2 glycoprotein and it may not be directly involved in antigen synthesis. However, substitution of Ala110Thr has been reported as the cause of the paraBombay phenotype in a secretor individual of Chinese origin[16] so this region can be sensitive to amino-acid substitutions. Surprisingly, despite the absence of H antigen, anti-B could be adsorbed onto and eluted from the red blood cells of donor #3, suggesting that the encoded fucosyltransferase may synthesise very low levels of H antigen that are converted to B antigen by the donor's B-transferase. It may therefore be more accurate to define this donor's phenotype as para-Bombay of the B_h type. However, this is somewhat paradoxical given the very high titre of anti-H in this donor's serum which is more consistent with the true O_h phenotype.

The glycine residue at position 320 (encoded by c.958G) in the C-terminus of the enzyme is highly conserved across homologous fucosyltransferases of many eukaryotic species, so it is likely that the substitution of the small, uncharged glycine to the much larger, positively charged arginine inactivates the transferase. Interestingly, this mutation has been deposited in dbRBC[15]: it was reported to have been found in a person of Chinese descent with the paraBombay phenotype, although no details of the person's phenotype are available.

One of other mutations described in this paper (p.Met1Leu), was originally reported by us in abstract form already in 2011[17], as the first example of a change expected to affect the initiation of protein synthesis from *FUT1*. The next available downstream methionine

Table III - Mutations in *FUT1* and *FUT2* genes in three individuals with the O_h phenotype.

		FUT1		FUT2	ABO genotype
Nt. position	1	323	958	428	
Consensus	A	G	G	G	
Donor #1	A	G	**A**	**A**	*ABO*O.01.01/01.01*
Donor #2	**C**	G	G	**A**	*ABO*O.01.01/02.01*
Donor #3	A	T	G	**A**	*ABO*O.01.01/02.01*
Amino acid position	1	108	320	428	
Consensus amino acid	Met	Arg	Gly	Trp	
Amino acid change	**Leu**	**Leu**	**Arg**	**Stop**	
Effect on function					
PolyPhen Score	0.779	0.984	1.00		
Conclusions	Possibly damaging	Probably damaging	Probably damaging		
SIFT	0	0.28	0.02		
Conclusions	Damaging	Tolerated	Damaging		

residue in this case is found at position 62 and, without expression studies, we cannot know whether this codon is used to synthesise an N-terminally truncated protein. However, the serological findings are consistent with the complete absence of both *FUT1*- and *FUT2*-derived α-1,2-fucosyltransferase activity. There are parallels with the ABO glycosyltransferase in which interruption of the start codon has been shown to be the mechanism responsible for a considerably less effective A-transferase resulting in an Aweak phenotype[18-20]. However, in the A transferase, there are methionine residues at positions 20, 26, 43, 53, and 69 which could potentially serve as alternative start codons. Expression studies in HeLa cell culture have demonstrated that weakly active enzymes are produced by constructs in which the initiation codon at position 1 is absent[19]. However, N-truncated ABO transferases have not yet been shown to exist. Recently, the translation-initiating mutation in *FUT1* was also reported in another individual of western European origin[21]. Contrary to our results, in their case antigen H was weakly expressed. In spite of this fact, these authors defined this *FUT2*-silent individual as having the "Bombay" phenotype.

Conclusions

We describe novel *FUT1* mutations in three unrelated blood donors of Polish origin. This provides further information to expand the mutational map of diversity for the *FUT1* gene and will facilitate interpretation of next-generation sequencing and similar data for blood group prediction in the future. In analogy with ABO, these and other inactivating or weakening mutations will also help draw a functional map of the fucosyltransferase once its three-dimensional structure is resolved.

Authorship contributions

Serological and molecular analyses were performed by GN, JW, AKH, AO, KG, EB and JRS. Data were interpreted and conclusions drawn by all authors. The manuscript was written by BM, MLO, EB and JRS. All authors contributed to and approved the final paper.

Keywords: blood group antigens, immunohaematology, blood group genetics, *FUT1*, Bombay.

The Authors declare no conflicts of interest.

References

1) Daniels G. *Human Blood Groups*. 3rd ed. Oxford: Wiley-Blackwell; 2013.
2) Reid ME, Lomas-Francis C, Olsson ML. *The Blood Group Antigen FactsBook*. London: Academic Press, 2012.
3) Wagner FF, Flegel WA. Polymorphism of the h allele and the population frequency of sporadic nonfunctional alleles. Transfusion 1997; **37**: 284-90. [Erratum: Transfusion 1997; **37**: 674].
4) Kelly RJ, Ernst LK, Larsen RD, et al. Molecular basis for H blood group deficiency in Bombay (Oh) and para- Bombay individuals. Proc Natl Acad Sci USA 1994; **91**: 5843-7.
5) Koda Y, Soejima M, Johnson PH, et al. Missense mutation of FUT1 and deletion of FUT2 are responsible for Indian Bombay phenotype of ABO blood group system. Biochem Biophys Res Commun 1997; **238**: 21-5.
6) Kelly RJ, Rouquier S, Giorgi D, et al. Sequence and expression of a candidate for the human Secretor blood group alpha(1,2) fucosyltransferase gene (FUT2). Homozygosity for an enzyme-inactivating nonsense mutation commonly correlates with the non-secretor phenotype. J Biol Chem 1995; **270**: 4640-9.
7) Judd WJ, Johnson ST, Storry JR. *Judd's Methods in Immunohematology*. Bethesda, USA: AABB Press; 2008.
8) Hult AK, Olsson ML. Many genetically defined ABO subgroups exhibit characteristic flow cytometric patterns. Transfusion 2010; **50**: 308-23.
9) Liu QP, Sulzenbacher G, Yuan H, et al. Bacterial glycosidases for the production of universal red blood cells. Nat Biotechnol 2007; **25**: 454-64.

10) Olsson ML, Chester MA. A rapid and simple ABO genotype screening method using a novel B/O 2 versus A/O 1 discriminating nucleotide substitution at the ABO locus. Vox Sang 1995; **69**: 242-7.
11) Olsson ML, Chester MA. Evidence for a new type of O allele at the ABO locus, due to a combination of the A 2 nucleotide deletion and the A el nucleotide insertion. Vox Sang 1996; **71**: 113-7.
12) Olsson ML, Chester MA. Polymorphism and recombination events at the ABO locus: a major challenge for genomic ABO blood grouping strategies. Transfus Med 2001; **11**: 295-313.
13) Hosseini-Maaf B, Hellberg A, Chester MA, Olsson ML. An extensive polymerase chain reaction-allele-specific polymorphism strategy for clinical ABO blood group genotyping that avoids potential errors caused by null, subgroup and hybrid alleles. Transfusion 2007; **47**: 2110-25.
14) Storry JR, Johannesson JS, Poole J, et al. Identification of six new alleles at the FUT1 and FUT2 loci in ethnically diverse individuals with Bombay and Para-Bombay phenotypes. Transfusion 2006; **46**: 2149-55.
15) Blumenfeld OO, Patnaik SK. Allelic genes of blood group antigens: a source of human mutations and cSNPs documented in the Blood Group Antigen Gene Mutation Database. Hum Mutat 2004; **23**: 8-16.
16) Guo Z, Xiang D, Zhu Z, et al. Analysis on FUT1 and FUT2 gene of 10 para-Bombay individuals. Vox Sang 2004; **87**: 81.
17) Michalewska B, Olsson ML, Naremska G, et al. New FUT1 mutations responsible for the H-deficient Bombay phenotype: the first example of an abolished start codon. Vox Sang 2011; **101** (suppl 1): 263.
18) Hult AK, Yazer MH, Jorgensen R, et al. Weak A phenotypes associated with novel ABO alleles carrying the A2-related 1061C deletion and various missense substitutions. Transfusion 2010; **50**: 1471-86.
19) Seltsam A, Das GC, Bade-Doeding C, Blasczyk R. A weak blood group A phenotype caused by a translation-initiator mutation in the ABO gene. Transfusion 2006; **46**: 434-40.
20) Cai X, Jin S, Liu X, et al. Molecular genetic analysis of ABO blood group variations reveals 29 novel ABO subgroup alleles. Transfusion 2013; **53**: 2910-6.
21) Letizia G, Vrignaud C, Peyrard T. Disruption of the start codon of the FUT1 gene is the molecular background of the first reported case of European non-secretor "Bombay" phenotype with a weak H antigen expression. Vox Sang 2015; **109**: 278-9.

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